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Identification of APOBEC3DE as Another Antiretroviral Factor from the Human APOBEC Family[▽]

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A tandem arrayed gene cluster encoding seven cytidine deaminase genes is present on human chromosome 22. These are APOBEC3A, APOBEC3B, APOBEC3C, APOBEC3DE, APOBEC3F, APOBEC3G, and APOBE C3H. Three of them, APOBEC3G, APOBEC3F, and APOBEC3B, block replication of human immunodeficiency virus type 1 (HIV-1) and many other retroviruses. In addition, APOBEC3A and APOBEC3C block intracellular retrotransposons and simian immunodeficiency virus (SIV), respectively. In opposition to AP OBEC genes, HIV-1 and SIV contain a virion infectivity factor (Vif) that targets APOBEC3F and APOBEC3G for polyubiquitylation and proteasomal degradation. Herein, we studied the antiretroviral activities of the human APOBEC3DE and APOBEC3H. We found that only APOBEC3DE had antiretroviral activity for HIV-1 or SIV and that Vif suppressed this antiviral activity. APOBEC3DE was encapsidated and capable of deaminating cytosines to uracils on viral minus-strand DNA, resulting in disruption of the viral life cycle. Other than GG-to-AG and AG-to-AA mutations, it had a novel target site specificity, resulting in introduction of GC-to-AC mutations on viral plus-strand DNA. Such mutations have been detected previously in HIV-1 clinical isolates. In addition, APOBEC3DE was expressed much more extensively than APOBEC3F in various human tissues and it formed heteromultimers with APOBEC3F or APOBEC3G in the cell. From these studies, we concluded that APOBEC3DE is a new contributor to the intracellular defense network, resulting in suppression of retroviral invasion.

APOBEC (*apo*lipoprotein *B* mRNA-*e*diting *c*atalytic polypeptide) proteins are a group of cytidine deaminases, which include APOBEC1 (A1), AID, APOBEC2 (A2), and a subgroup of APOBEC3 (A3) proteins in humans (22). Recently, another poorly expressed human APOBEC protein, APOB EC4, was identified (45). All of these proteins contain one or two Zn^{2+}-binding motifs ($HxEx_{23-28}PCx_{2-4}C$), which catalyze cytidine deamination (34, 63). These proteins target cytosines and convert them to uracils (C to U) on a variety of vertebrate-specific RNA/DNA targets (41, 57). In fact, the field of retroviral research has been spurred by the discovery that such cytidine deamination could block retroviral replication.

On human chromosome 22, there is a cluster of tandem arrayed A3 genes. Originally, they were designated A3A to A3H (22, 60). Later, it was found that A3D and A3E are two regions of the same molecule (11). Thus, there are seven A3 genes, which are A3A, A3B, A3C, A3DE, A3F, A3G, and A3H. A3A, A3C, and A3H have one and A3B, A3DE, A3F, and A3G have two Zn^{2+}-binding motifs. In sharp contrast, only a single A3 gene (mA3), which produces a protein with two Zn^{2+}-binding motifs (60), was found in mice. The function of the A3 genes remained unknown until it was discovered that human A3G inhibits human immunodeficiency virus type 1 (HIV-1) replication (49). Indeed, similarly to A3G, human A3F and A3B were found to inhibit the replication of HIV-1 (2, 30, 61, 72) and many other viruses, such as simian immunodeficiency virus (SIV) and human hepatitis B virus (12); human A3C blocked SIV but not HIV-1 (64); and human A3A blocked replication of the adeno-associated virus and retrotransposons such as intracisternal A particle (IAP) and long interspersed element 1 (LINE-1) (4, 5, 9). Very recently, two groups studied the functions of human A3DE and A3H, respectively, and found that they did not exhibit any antiretroviral activities (44, 52). Nonetheless, since genetic knockout of the A3 gene did not affect mouse development, survival, or fertility, A3 genes might have evolved solely with an antiviral function (40).

A key step for A3 genes in blocking viral replication is their encapsidation. For example, murine leukemia virus (MLV) was restricted by human A3B and A3G but not by A3F or murine A3 (mA3) because MLV did not encapsidate either human A3F or mA3 (15, 26). Human A3B, A3F, and A3G incorporation into HIV-1 is due to their interactions with Gag proteins in the nucleocapsid region, although it is still unclear whether these interactions are mediated by cellular or viral genomic RNAs (1, 8, 14, 16, 25, 27, 32, 47, 56, 69). Following entry into the target cell, the virus starts uncoating and the ensuing reverse transcription results in synthesis of viral minus-strand cDNA. During this process, A3 genes deaminate dC to form dU on newly synthesized minus-strand viral cDNAs, causing G-to-A mutations on the plus-strand DNA and abortive infection (20, 29, 35, 71). Nonetheless, the viral protein Vif counters the antiviral activities of A3F and A3G by blocking their encapsidation. Vif binds specifically to A3F and A3G (but not to A3B) (14, 38, 61) and also interacts with a protein complex containing ElonginB, ElonginC, and Cullin5 (Cul5), which are the core subunits of Cul5-based E3 ubiquitin ligases,

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such as SCF (66). SCF belongs to the RING (*r*eally *i*nteresting *n*ew *g*ene) family E3 ubiquitin ligases, which normally contain RING finger proteins, such as Ring box 1 (Rbx1), as another enzymatic component to recruit E2 ubiquitin ligases. Indeed, Vif has a BC box motif (^{144}S^{145}L^{146}Q) that binds to ElonginC (39, 67) and an HCCH motif (^{114}C/^{133}C) that binds to Cul5 (33). Thus, Vif is able to bridge the Cul5-based E3 ligase complex to A3F and A3G and trigger their degradation in the 26S proteasome (31, 38, 50, 53, 72). As a consequence, A3 proteins are excluded from virions and unable to block viral replication.

Interestingly, the activity of Vif is highly species specific. For example, Vif from HIV-1 blocked A3G from humans and chimpanzees but not from monkeys, Vif from SIV isolated from African green monkey (SIVagm) blocked only A3G from the same species, and Vif from SIV isolated from rhesus macaque (SIVmac) blocked A3G from human and nonhuman primates (37). Indeed, a genetic study revealed that a single residue in human A3G, D128, determined its sensitivity to HIV-1 Vif. This was discovered by the fact that Vif from HIV-1 did not bind to A3G from African green monkey (agmA3G) because agmA3G contains a lysine (K) rather than an aspartate (D) at position 128. When this residue was exchanged between human A3G and agmA3G, human A3G became resistant and agmA3G became sensitive to HIV-1 Vif (3, 36, 48, 62).

In this report, we studied antiretroviral activities of the human A3DE and A3H proteins. As previously reported (44), we did not detect any antiretroviral activity of A3H. However, unlike another previous report (52), we found that A3DE blocked the replication of both HIV-1 and SIV but not that of MLV, although its effect was weaker than those of A3F and A3G. The antiretroviral activity of A3DE was accomplished by the encapsidation of A3DE and the resulting mutations of newly synthesized viral minus-strand DNA. A3DE and A3G were expressed much more extensively and broadly than A3F in those nonpermissive cells and tissues, where they might form an intermolecular complex to combat HIV-1 infection. Although Vif counteracted the activity of A3DE, it is clear that A3DE is a new contributor to the intracellular defense network that sets another hurdle to retroviral invasion.

MATERIALS AND METHODS

Plasmids. HIV-1 proviral constructs pNLΔVif, pNL-Luc, and pNL-LucΔVif and mammalian expression vectors for human AID, A1, A2, A3C, A3F, and A3G and mA3 were described before (72). Human A3A and A3B expression vectors were obtained from M. H. Malim (2) and were subcloned into pcDNA3.1D/V5-His-TOPO (Invitrogen, Carlsbad, CA). cDNA clones for human A3DE and A3H were obtained commercially (Open Biosystems, Huntsville, AL), and these genes were subcloned into the same vector. All of these APOBEC proteins contained a V5 tag at their C termini. To create plasmids expressing A3 and glutathione *S*-transferase (GST) or luciferase fusion proteins, GST and firefly luciferase genes were amplified from pGEX-6T1 (Amersham, Piscataway, NJ) or pSP-*Luc*+NF (Promega, Madison, WI) by PCR and inserted into these A3 expression vectors by XbaI and NotI digestion. This cloning did not interfere with the expression of the C-terminal V5 tag. To measure MLV infectivity, the firefly luciferase gene was cloned into the pLNCX2 vector (BD Biosciences, Palo Alto, CA) by XhoI and NotI digestion. The Vif expression vector pNL-A1 (55) and its control, pNL-A1ΔVif (23), were obtained from K. Strebel. The SIV proviral clones SIVmac-Luc, SIVmac-LucΔVif, SIVagm-Luc, and SIVagm-LucΔVif were obtained from N. R. Landau (37). The expression vectors for Cul5ΔNedd and Cul5ΔRbx were obtained from B. Liu and X. Yu (66). The cytomegalovirus-driven MLV *gag-pol* expression vector pCgp was obtained from Paula Cannon. The A3DE$_{D140K}$, A3F$_{E127K}$, and A3G$_{D128K}$ mutants were created with a QuikChange XL site-directed mutagenesis kit (Stratagene, La Jolla, CA).

Viral production and infectivity assay. HIV-1, SIVmac, SIVagm, and MLV virions were produced from 293T cells by a standard calcium phosphate transfection. Typically, 25 μg of total plasmid DNA was used for each transfection in a 100-mm culture dish with 40% confluence of cells. Both SIVmac and MLV were pseudotyped with vesicular stomatitis virus glycoprotein (VSV-G) by cotransfection of 5 μg VSV-G expression vector because they did not encode a functional envelope protein. The ratio between these proviral constructs and A3 expression vectors was normally kept at 1:4. For example, MLV was produced by cotransfection of 2 μg pCgp, 2 μg pLNCX2-Luc, 5 μg VSV-G, and 16 μg A3. The production of HIV-1 and SIVmac was quantitated by p24^{Gag} or p27^{Gag} capture enzyme-linked immunosorbent assay, respectively. The production of SIVagm and MLV was quantitated by viral reverse transcriptase (RT) activity, and the reaction cocktail contained 50 mM Tris-HCl, pH 8.0, 75 mM KCl, 2 mM dithiothreitol, 24 μg/ml poly(rA) · oligo(dT), 5 mM $MgCl_2$ (for SIV) or $MnCl_2$ (for MLV), 1% NP-40, and 100 μCi/ml [^{3}H]dTTP (18). In some experiments, viruses were further purified by being loaded onto a 20% sucrose cushion and spun at 27,000 rpm for 2 h in a Beckman SW28 rotor and viral particles were resuspended in STE buffer (10 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM EDTA). Equal amounts of viruses were used to infect GHOST-R3/X4/R5 cells. Thirty-six hours later, cells were lysed in a buffer (25 mM Tris-HCl, pH 7.8, 2 mM dithiothreitol, 2 mM 1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid, 10% glycerol, 1% Triton X-100). After removing the nuclei, the cytosolic fraction was used to determine the luciferase activity with a luciferase assay kit (Promega).

Sequencing of newly synthesized viral cDNA and real-time PCR quantitation. GHOST-R3/X4/R5 cells were infected with various HIV viruses produced from 293T cells, which were passed through a 0.22-μm filter unit (Millipore, Bedford, MA) and treated with RQ1 RNase-free DNase (Promega) to remove any plasmid DNA contamination. Several hours later, cellular DNAs were extracted with a DNeasy tissue kit (QIAGEN Inc., Valencia, CA). A 420-bp fragment was PCR amplified by a previously described primer pair (72) and cloned into the pCR4-TOPO vector (Invitrogen). Multiple clones were selected and sequenced by the flanking T3 and T7 primers. Alternatively, the newly synthesized viral cDNA was quantitated by a previously described real-time PCR method with primer pair MH531 and MH532 and probe LRT-P (7).

A3DE mRNA expression analysis by RT-PCR. TRIzol (Invitrogen) was used to isolate total cellular RNAs from various permissive or nonpermissive cell lines. Total RNA from human peripheral blood leukocytes (PBL) was purchased from Clontech (Mountain View, CA). The primer pairs for A3DE and A3F were 5′-CGGTACTACCCAAACGTCAGTCGAATCACAGGCA-3′/5′-AGGAGCACCCACCGC-3′ and 5′-GCAAAGATCTTTCGAGGCCAG-3′/5′-ATGTGTGGATACATTGCCT-3′, respectively. The primer pair for A3G was described before (2). The primer pair for GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was 5′-CGGAGTCAACGGATTTGGTCGTAT-3′/5′-AGCCTTCTCCATGGTGGTGAAGAC-3′. Five micrograms total RNA was subjected to reverse transcription using SuperScript II reverse transcriptase (Invitrogen) and an oligo(dT) primer according to the manufacturer's instructions. Prior to the reverse transcription step, all RNAs were treated with RQ1 RNase-free DNase (Promega) to remove any genomic DNA contamination. In addition, a PrimeExpress II human normal tissue cDNA panel containing high-quality, PCR-ready, first-strand cDNAs from 30 different human tissues was purchased (Primgen, Bothell, WA). These cDNAs were then subjected to 35 PCR cycles at the following temperatures for the indicated lengths of time: 94°C for 45 s; 55°C for 45 s; and 72°C for 90 s. PCR products were then resolved by 1.0% agarose gel and visualized by ethidium bromide staining.

In vivo GST pulldown assay. 293T cells were transfected with various vectors expressing GST or GST fusion and Vif proteins and lysed with radioimmunoprecipitation assay buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, 5 μg/ml aprotinin, 5 μg/ml leupeptin). The cytosolic fraction was precleared with Sepharose 4B beads and then rocked with prewashed glutathione (GSH)-Sepharose beads for 2 h at 4°C. After extensive washing with phosphate-buffered saline, bead-associated proteins were analyzed by Western blotting.

Western blotting. Horseradish peroxidase (HRP)-conjugated anti-V5 antibody (Invitrogen) was used to directly detect the expression of A3, GST, A3-GST, and A3-Luc proteins. Actin was detected by a polyclonal antibody (C-11) (Santa Cruz Biotechnology, Santa Cruz, CA), and HIV-1 p24^{Gag} and Vif were detected by two monoclonal antibodies (no. 3537 and no. 6459) from the NIH AIDS Research and Reference Reagent Program. HRP-conjugated anti-rabbit or -mouse immunoglobulin G secondary antibodies were from Pierce (Rockford, IL). De-

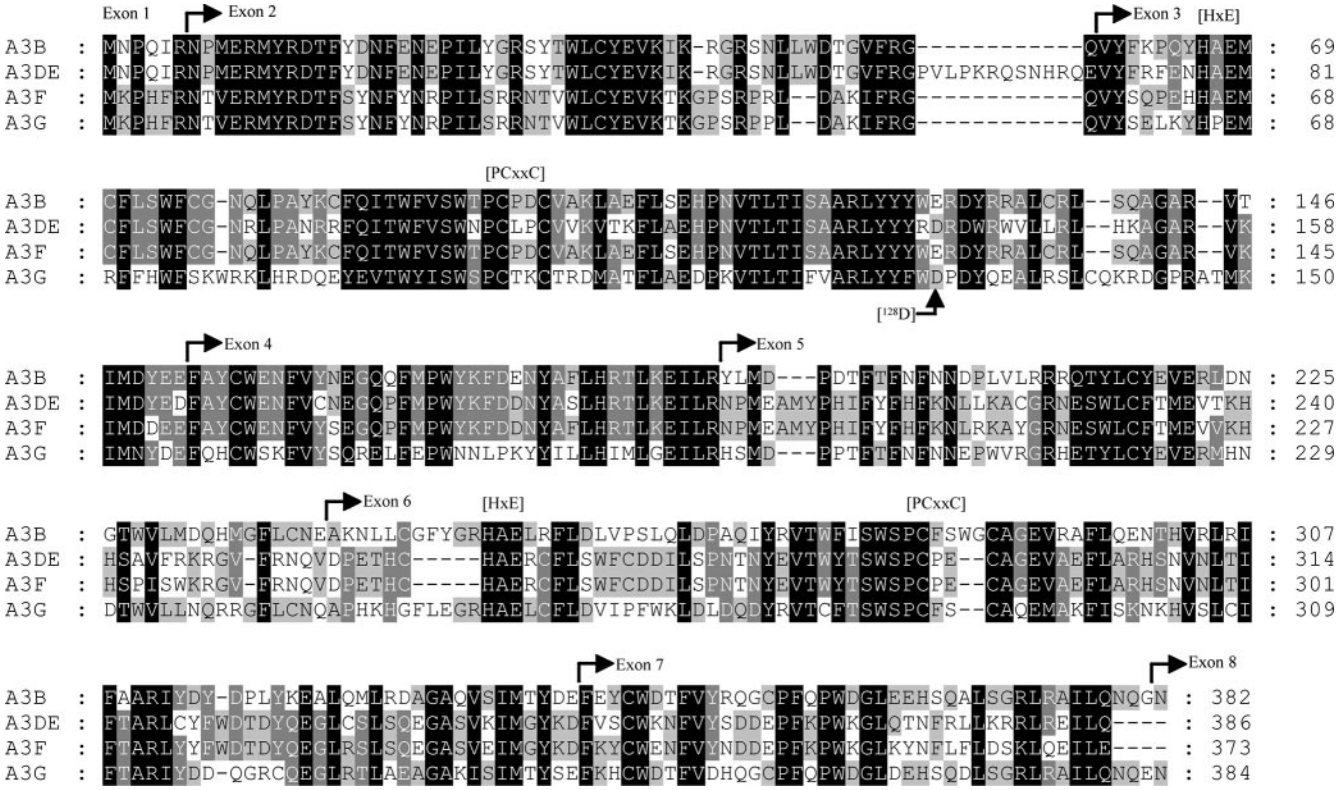

FIG. 1. Amino acid sequence alignment of human A3B, A3DE, A3F, and A3G. Conserved residues are shown against a black background and partially conserved residues are shown against a grey background. Dashes indicate deletions. The key motif for Zn^{2+}-binding motif HxE/PCxxC is indicated. D128, which determines the species-specific interaction between A3G and Vif, is indicated by an arrow below the sequences. Eight exon positions are indicated. Intron-exon boundary information for A3B, A3F, and A3G was obtained from a previous report (22), and that for A3DE was obtained from the Ensembl database.

tection of the HRP-conjugated antibody was performed using an enhanced chemiluminescence detection kit (Amersham Bioscience, Pittsburgh, PA).

RESULTS

Human A3DE and A3H genes. To obtain human A3 cDNAs, we performed a BLAST search of the Ensembl database (http://www.ensembl.org) and obtained cDNA sequences for A3DE (Ensembl gene ID ENSG00000179007) and A3H (Ensembl accession no. ENSG00000100298). These sequences were used to search the cDNA clone distributor's database (http://www.openbiosystems.com), and two clones that contained the full-length A3DE or A3H coding sequence, respectively, were obtained. Sequence analyses showed that A3DE shares 76.9% amino acid sequence homology with A3F, 51.7% homology with A3B, and 41.7% homology with A3G. As presented in Fig. 1, both of the N- and C-terminal Zn^{2+}-binding motifs (HxE/PCxxC) and the aspartate (D128) that determines A3G specificity to Vif are all well conserved in these proteins. Whereas A3F and A3G differ by a single amino acid from residues 1 to 61, A3DE and A3B are identical from residues 1 to 57. In addition, A3DE and A3F share 100% homology in exon 5, and they differ by only 3 amino acids in exon 6, where the second Zn^{2+}-binding domain is located. Thus, the N-terminal half of A3DE has very high sequence identity with that of A3B, and the C-terminal half of A3DE has very high sequence identity with that of A3F. In contrast, A3H is much less homologous to A3A and A3C. It shares only 29.6% and 34.7% amino acid sequence with A3A and A3C, respectively, although they all have a highly conserved HxE/PCxxC motif (data not presented).

Human A3DE blocks HIV-1 replication, but Vif counters its activity. The genomic similarity of A3DE and A3H to previously characterized A3 proteins provides a compelling case for determining their roles in ablating HIV-1 replication. A3DE and A3H were subcloned into the pcDNA3.1 vector, and their anti-HIV activities were compared with those of human A3A, A3B, A3C, A3F, and A3G proteins. To simplify the measurement of HIV infectivity, the wild-type HIV-1 luciferase reporter virus (pNL-Luc) and its Vif deletion version (pNL-LucΔVif) were used as described before (72). These plasmids were cotransfected into 293T cells with various combinations to produce viruses. As presented in Fig. 2A (bottom panel), all human A3 proteins were expressed in viral producer cells at the predicted sizes. Previously, lower-molecular-weight products associated with A3G were detected from cell lysates (37). We not only confirmed this observation but also detected similar lower-molecular-weight products from A3B- and A3F-transfected cells. However, it was unclear how these A3 proteins were cleaved.

Equal amounts of viruses were then collected to infect GHOST cells, and levels of intracellular luciferase activities were measured. As presented in Fig. 2A (top panel), A3A and

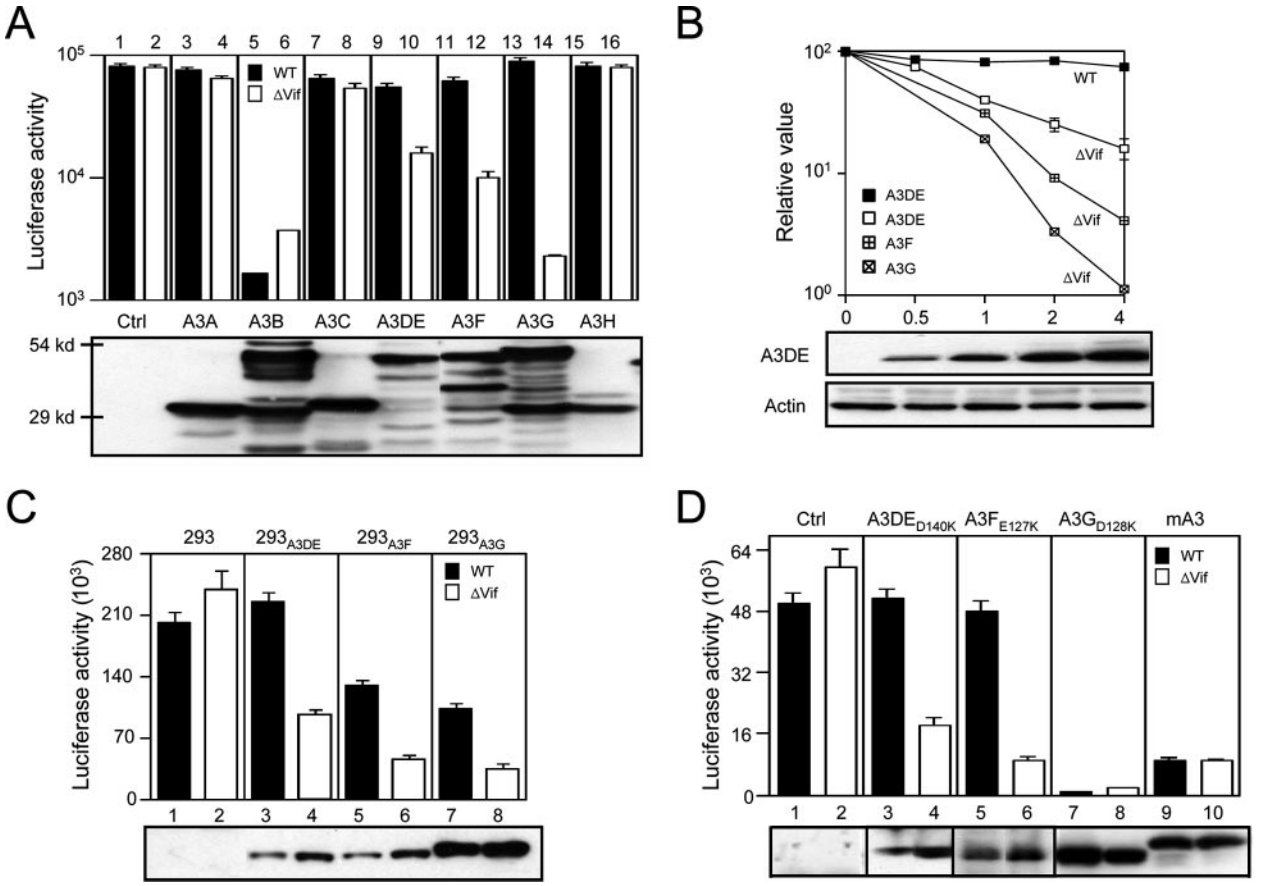

FIG. 2. Human A3DE blocks HIV-1 replication, and its activity is countered by Vif. (A) A3DE anti-HIV-1 activity. Human A3A, A3B, A3C, A3DE, A3F, A3G, and A3H were cotransfected with HIV-1 reporter provirus pNL-Luc or pNL-LucΔVif in 293T cells, and viruses were collected from cell culture supernatants. After normalization to the levels of $p24^{Gag}$, these viruses were used to infect GHOST.X4/R5 cells. Thirty-six hours later, cells were lysed and intracellular luciferase activities were measured. The bottom panel shows protein expression levels of these A3 proteins in viral producer cells (293T) as determined by Western blotting (Ctrl, control vector). (B) Inhibition of Vif-defective HIV-1 by A3DE is dose dependent. The pNL-Luc or pNL-LucΔVif proviral construct was cotransfected into 293T cells with increasing amounts of A3DE, A3F, and A3G expression vector. Viruses were collected and analyzed similarly to the procedure described above. The bottom panel shows A3DE expression levels in viral producer cells as determined by Western blotting. (C) Stable expression of A3DE changes 293 cells from a permissive to a nonpermissive cell type. 293 cells were transfected with linearized A3DE, A3F, or A3G expression vector, and cells were selected by culture medium containing 50 μg/ml G418 to establish stable cell lines. Wild-type (WT) or Vif-defective (ΔVif) HIV-1 was then produced from these cells, and viral infectivity was determined. The bottom panel shows protein expression levels of A3DE, A3F, and A3G proteins in these cells as determined by Western blotting. (D) A previously identified single residue of A3G, D128, does not govern A3DE sensitivity to Vif of HIV-1. The consensus residue in A3DE (D140), A3F (E127), or A3G (D128) was mutated to lysine (K), and anti-HIV activities were determined as described for panel A. The bottom panel shows protein expression levels of these mutants ($A3DE_{D140K}$, $A3F_{E127K}$, and $A3G_{D128K}$ mutants) in viral producer cells as determined by Western blotting. Error bars represent the standard deviations from at least three independent experiments.

A3C did not block either wild-type or Vif-deficient HIV-1 (Fig. 2A, lanes 3, 4, 7, and 8), A3F and A3G selectively blocked Vif-deficient HIV-1 (Fig. 2A, lanes 11 to 14), and A3B blocked both wild-type and Vif-deficient HIV-1 (Fig. 2A, lanes 5 and 6). Interestingly, A3DE blocked the replication of the Vif-deficient HIV-1 but not that of the wild-type virus, albeit its effect was not as strong as those of A3B, A3F, and A3G (Fig. 2A, lanes 9 and 10). In contrast, A3H did not block either wild-type or Vif-deficient HIV-1 (Fig. 2A, lanes 15 and 16). To further confirm the effect of A3DE, we titrated its antiretroviral activity. As presented in Fig. 2B, when the amounts of A3DE expression vector were increased from 0 to 0.5, 1, 2, and 4 μg during cotransfection with HIV-1 proviral constructs, the intracellular expression of A3DE increased proportionally. Importantly, infectivity of only the Vif-defective but not of the wild-type HIV-1 was substantially decreased by the dose-dependent increase of A3DE expression, which further confirmed that A3DE blocked replication of only the Vif-defective HIV-1. As a comparison, we also titrated A3F and A3G anti-HIV-1 activities in a similar setting. At the highest concentration, A3DE, A3F, and A3G reduced Vif-deficient HIV-1 infectivity by 7-, 25-, and 100-fold, respectively (Fig. 2B), which further confirmed that the anti-HIV-1 activity of A3DE was lower than those of A3F and A3G.

Next, we wanted to test whether A3DE blocks HIV-1 replication when it is expressed endogenously. We generated 293 cell lines stably expressing A3DE, A3F, and A3G (Fig. 2C, bottom panel). Typically, A3 protein expression levels in these stably transfected cells were at least fivefold lower than those in transiently transfected cells (data not shown). Even under

A

Summary of HIV-1 mutations by A3G and A3DE

	A3G		A3DE	
	WT	ΔVif	WT	ΔVif
Total clones (base pairs)	25 (10275)	19 (7809)	20 (8220)	104 (42744)
A-to-G	4	6	1	25
A-to-C				5
A-to-T	1		1	
G-to-C	1			1
C-to-T	1	22	1	5
T-to-A	1			8
T-to-G		1		
T-to-C	1	9	2	25
G-to-A	**0**	**39**	**0**	**57**
Total mutations	9	**77**	**5**	**126**
Mutation rate (%)	0.08	0.98	0.06	0.30
G-to-A mutation rate (%)	0	0.5	0	0.13

B

A3DE consensus editing sequence

	-3	-2	-1	0	+1
A	18	33	46		5
C	37	14	16	100	25
G	9	19	4		35
T	37	33	35		35
Total (%)	100	100	100	100	100
Consensus	C/T	T/A	T/A	C	G/T
A3F Consensus (Ref.61)	?	T	T	C	T
A3G Consensus (Ref.61)	?	C	C	C	A

C

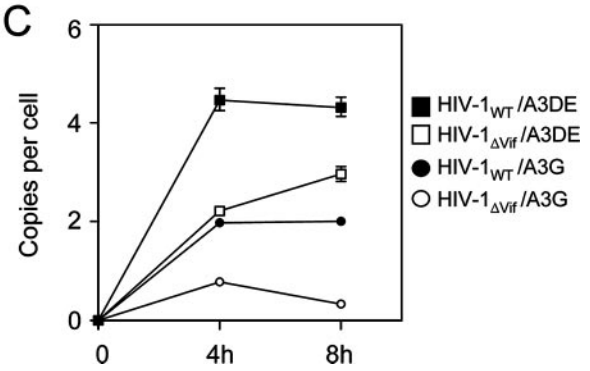

FIG. 3. A3DE induces C-to-U hypermutations on viral minus-strand transcripts that are degraded in the target cell. (A) Summary of HIV-1 genome mutations introduced by A3DE and A3G. Wild-type (WT) or Vif-defective (ΔVif) HIV-1 produced in the presence of A3DE or A3G was used to infect GHOST cells. Several hours later, cellular DNAs were extracted and viral DNAs were amplified by PCR. The PCR products were then cloned into the TA cloning vector and sequenced. (B) Summary of A3DE target site sequence specificity. Results from 57 G-to-A mutations in the genome of Vif-deficient HIV-1 introduced by A3DE were used for this analysis. (C) Stability of newly synthesized HIV-1 reverse transcripts. Wild-type and Vif-defective HIV-1 were produced in the presence of A3DE or A3G, and these viruses were used to infect GHOST cells. Four or eight hours later, cellular DNAs were extracted from these infected cells, and newly synthesized viral cDNA was quantified by quantitative real-time PCR with primers specific for the late reverse transcripts (see Materials and Methods). Error bars represent the standard deviations from at least three independent experiments.

conditions of such low expression, A3DE as well as A3F and A3G was able to reduce infectivity of Vif-deficient HIV-1 when viruses were produced from A3-expressing cells (Fig. 2C, top panel).

As noted earlier, a single residue (D128) governs the sensitivity of human A3G to Vif (3, 36, 48, 62). This residue is conserved in A3F (E127) and A3DE (D140). To determine whether it governs A3DE sensitivity to Vif, we mutated it to lysine ($A3DE_{D140K}$ mutant) and similar A3F and A3G mutants were also generated as controls ($A3F_{E127K}$ and $A3G_{D128K}$ mutants). Similarly to murine A3 (mA3), which is not sensitive to Vif, $A3G_{D128K}$ blocked the replication of both wild-type and Vif-defective HIV-1 (Fig. 2D, lanes 5 to 8); however, $A3DE_{D140K}$ and $A3F_{E127K}$ were still unable to block the wild-type virus (Fig. 2D, lanes 1 to 4). This result indicates that, unlike in A3G, this conserved residue itself is not sufficient to determine the sensitivities of both A3DE and A3F to Vif.

A3DE induces a G-to-A hypermutation in newly synthesized viral cDNA with novel target specificity. Having found that A3DE blocked Vif-defective HIV-1 replication, we wanted to know whether cytidine deamination was involved for such anti-HIV-1 action. To test this, GHOST-R3/X4/R5 cells were infected with either wild-type or Vif-defective HIV-1 produced from 293T cells in the presence of A3DE. For comparison, human A3G was also included in this study. At 4 and 8 h of viral infection, cellular DNAs were extracted and newly synthesized viral cDNA was amplified by PCR with a primer pair encompassing the Vif/Vpr region as described before (72). PCR products were cloned, and individual clones were sequenced. One hundred sixty-eight clones were sequenced (Fig. 3A), and we detected a high frequency of G-to-A mutations in HIV-1 plus-strand DNA from samples infected with Vif-deficient HIV-1 produced in the presence of either A3DE or A3G. No G-to-A mutations were detected from samples infected

with wild-type HIV-1 produced in the presence of either A3DE or A3G. The frequency of G-to-A mutations in the presence of A3G (0.5%) was higher than that in the presence of A3DE (0.13%), which might explain why A3G exhibited a higher anti-HIV-1 activity than A3DE. We also detected relatively high frequencies of other types of mutations, such as A-to-G, A-to-C, C-to-T, and T-to-C mutations, in these two samples. Because these mutations were present in all of the samples with similar frequencies, they were most likely errors introduced by viral reverse transcription and/or PCR. Thus, like A3G, A3DE introduced G-to-A hypermutations into the HIV-1 genome. Since the G-to-A mutation in viral plus-strand DNA corresponds to the C-to-U mutation in the minus-strand DNA, these results demonstrate that A3DE exhibits cytidine deamination enzymatic activity in the viral life cycle when Vif is not present.

We further analyzed 57 G-to-A mutations to reveal the consensus sequence for A3DE editing (Fig. 3B). As previously reported, A3G normally mutates C residues on the minus-strand DNA flanked at the −1 and −2 positions by C residues, resulting in GG-to-AG and GG-to-AA mutations, and A3F normally mutates C residues flanked at the −1 and +1 positions by T residues, resulting in GA-to-AA mutations on the plus-strand DNA (30, 61). For A3DE, the mutated C residues were flanked at the −1 position by either T or A residues and at the +1 position by either G or T residues on the minus-strand DNA, resulting in either GA-to-AA or GC-to-AC mutations on the plus-strand DNA. Since the GC-to-AC mutation has not been attributed to any other APOBEC family members, it might therefore be used as a genetic marker to trace A3DE activity in vivo.

Because uracil-containing viral cDNA might be degraded by cellular DNA repair enzymes (such as uracil DNA glycosylase and apurinic endonuclease), we next determined the effect of cytidine deamination on the fate of the newly synthesized viral cDNA by real-time PCR. The copy number of HIV-1 reverse transcripts was significantly decreased by both A3DE and A3G when Vif was absent (Fig. 3C). Thus, we conclude that cytidine deamination is a mechanism of A3DE-mediated antiretroviral activity.

Vif inhibits A3DE encapsidation by triggering proteasomal degradation. The finding that A3DE blocked Vif-deficient but not wild-type HIV-1 replication suggests that HIV-1 Vif suppresses A3DE's antiretroviral activity (Fig. 2). To explore this mechanism, we first determined whether the presence of Vif resulted in exclusion of A3DE from virion incorporation. Wild-type and Vif-defective HIV-1 were produced in the presence of A3DE and purified, and A3G was used as a control (Fig. 4A). Analysis of equal amounts of viral particles (as judged by $p24^{Gag}$ levels) showed that Vif-defective virions contained significantly greater levels of both A3DE and A3G proteins (Fig. 4A), which suggests that Vif excludes A3DE from virions. To define further how Vif excluded A3DE from virions, we compared the intracellular levels of A3DE in the presence and absence of Vif. Indeed, in cells expressing Vif, the levels of A3DE were significantly lower (Fig. 4A, middle panel, lane 1). Similarly, the levels of A3G were also decreased in cells expressing Vif (Fig. 4A, middle panel, lane 3). Thus, we conclude that Vif destabilizes both A3DE and A3G.

We next developed an in vivo GST pulldown assay to determine whether Vif could bind to A3DE in cells. We fused a GST tag to the C termini of A3A, A3DE, and A3G, and these chimeras were then coexpressed with Vif in 293T cells. Proteins were pulled down by GSH-Sepharose beads, and all proteins were expressed with the predicted sizes in cells (Fig. 4B, lanes 2, 4, 6, and 8). However, only A3DE-GST and A3G-GST chimeras were able to pull down Vif (Fig. 4B, lanes 5 and 7). Thus, we conclude that, like A3G, A3DE binds to Vif in vivo.

Finally, we determined whether Vif could trigger the degradation of A3DE in the 26S proteasome. The interaction between Vif and A3DE implies that A3DE may become another substrate for the Cul5-based E3 ligases. To detect such degradation, we devised a reporter system where the firefly luciferase gene (Luc) was fused to the C termini of A3 proteins (Fig. 4C). Our hypothesis was that if Vif triggers the degradation of A3DE, it will also trigger the degradation of A3DE-Luc fusion protein and therefore decrease cellular luciferase levels. Thus, by measuring the intracellular levels of luciferase, the levels of A3 protein degradation can be monitored. Furthermore, the dominant negative Cul5 constructs (Cul5ΔNedd8 and Cul5ΔRbx) that interfere with the assembly of Cul5-based E3 ligases should block the degradation of A3DE-Luc fusion. As presented in Fig. 4D, Vif did not reduce the levels of A3B-Luc luciferase activity (Fig. 4D, lane 2), which was consistent with the fact that Vif failed to block the A3B anti-HIV-1 activity (Fig. 2A, lane 5). However, Vif significantly reduced the levels of A3G-Luc luciferase activity (Fig. 4D, lane 8), which was consistent with the fact that A3G was very sensitive to Vif. In addition, expression of Cul5ΔRbx recovered the loss of A3G-Luc luciferase activity (Fig. 4D, lane 9). With regard to Vif-induced A3DE degradation, we found that Vif reduced the A3DE-Luc luciferase activity to a level similar to that noted for A3G-Luc (Fig. 4D, lane 4). Moreover, Cul5ΔNedd partially and Cul5ΔRbx completely reversed this reduction (Fig. 4D, lanes 5 and 6). Thus, we conclude that Vif recruits Cul5-based E3 ligases to trigger A3DE polyubiquitylation and degradation in the 26S proteasome, ultimately resulting in the absence of A3DE from the virion.

A3DE mRNA expression and its interaction with A3F and A3G. Human cell lines are defined as permissive or nonpermissive based on their ability to support Vif-defective HIV-1 infection (6). Vif-defective HIV-1 propagates only in permissive cells and not in nonpermissive cells (17, 59). Although A3B, A3F, and A3G can all block HIV-1 replication, only A3F and A3G were found to be expressed in nonpermissive T cells and tissues, including the primary targets for HIV-1 infection (2, 30, 61); A3B was barely detectable in any human cell lines or tissues (2, 14). These results suggest that A3F and A3G play a dominant role in defense of HIV-1 infection in these nonpermissive cells. However, due to the high sequence similarity between A3DE and A3F, we found that previously described primers that were used to detect A3F transcripts in different cells and tissues by RT-PCR also amplified A3DE transcripts (data not presented) (2, 14, 61). Thus, A3DE could be expressed in those tissues where A3F was detected. To clarify this ambiguity and understand precisely how A3DE and A3F are expressed in vivo, we designed two primer pairs to specifically amplify A3DE and A3F (Fig. 5A). For comparison, we used a previously described A3G-specific primer pair to amplify A3G transcripts (2) (Fig. 5A).

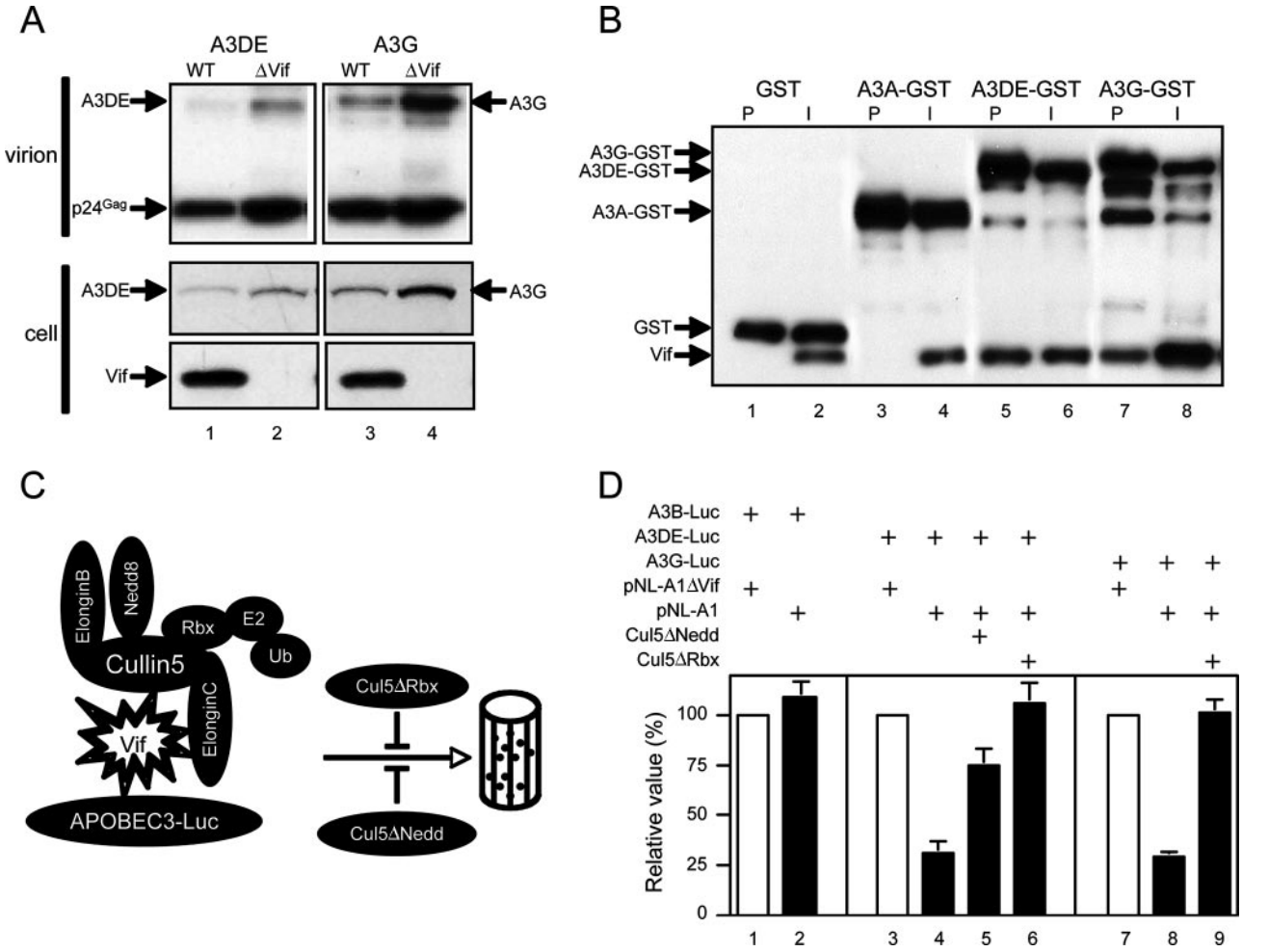

FIG. 4. Vif excludes A3DE from HIV-1 virions by destabilizing A3DE in viral producer cells. (A) Vif excludes A3DE from virions. 293T cells were cotransfected with wild-type (WT) or Vif-defective (ΔVif) HIV-1 proviral clones in the presence of A3DE and A3G. Virions were collected 48 h later and purified. Expression levels of A3, $p24^{Gag}$, and Vif in virions or viral producer cells were determined by Western blotting. (B) Vif binds to A3DE. A GST tag was fused to the C termini of A3A (A3A-GST), A3DE (A3DE-GST), and A3G (A3G-GST), followed by another V5 tag. These GST or GST fusion proteins were coexpressed with Vif in 293T cells, and proteins were then pulled down with GSH-Sepharose beads. Both input (I) and pulldown (P) samples were analyzed by Western blotting using antibodies against V5 or Vif. (C) Schematic representation of a reporter system detecting A3 protein stability in the presence of Vif. A firefly luciferase gene was fused to the C termini of A3 proteins. Vif recruits the Cul5-based E3 ubiquitin ligase complex to these A3-luciferase fusions for degradation in the 26S proteasome. The dominant negative Cul5ΔNedd mutant or Cul5ΔRbx mutant, which lacks Nedd8 or the Rbx binding site, respectively, disrupts the assembly of this E3 ligase complex and therefore blocks this degradation pathway. CMV, cytomegalovirus. (D) Vif destabilizes A3DE in cells. A3-luciferase fusions (A3B-Luc, A3DE-Luc, and A3G-Luc) were coexpressed with HIV-1 Vif (pNL-A1) or its control (pNL-A1ΔVif) in the presence of Cul5ΔRbx or Cul5ΔNedd, respectively, in 293T cells. Thirty-six hours later, cells were lysed and intracellular luciferase activity was measured. Results are presented as values relative to those obtained without Vif expression in cells, and error bars represent the standard deviations from at least three independent experiments.

Next, these primers were used to determine A3 expression in human cell lines. As presented in Fig. 5B, A3DE mRNAs were detected in nonpermissive cells, such as MT4, H9, PM1, Hut78, JM1, and CEM cells and primary PBL, and, conversely, were not detected in permissive cells, such as HeLa, 293T, Molt4, and SupT1 cells. Because PBL include $CD4^+$ T cells and monocytes, this result suggested that A3DE might play a key role in resisting HIV-1 infection during primary infection. When the expression levels of A3F and A3G were determined, we found that A3G showed the same expression profile as A3DE. However, the expression of A3F was relatively weak in the same nonpermissive cells, particularly in PBL. In addition, A3F was not detected in CEM or JM1 cells (Fig. 5B, lanes 10 and 11). This result suggests that A3F might not be expressed as extensively as was thought before. Paradoxically, we detected high expression levels of A3DE, A3F, and A3G in A3.01 and U937 cells, which belong to permissive cell groups that should not express any functional A3 proteins. Previously, Rose et al. made a similar observation (46). Thus, it could be possible that either these cells do not express certain unknown cellular cofactors to support these A3 proteins for functioning or these A3 proteins are not effectively encapsidated by HIV-1 from these cells. Nonetheless, it would be interesting to further study the function of A3 proteins in these cells.

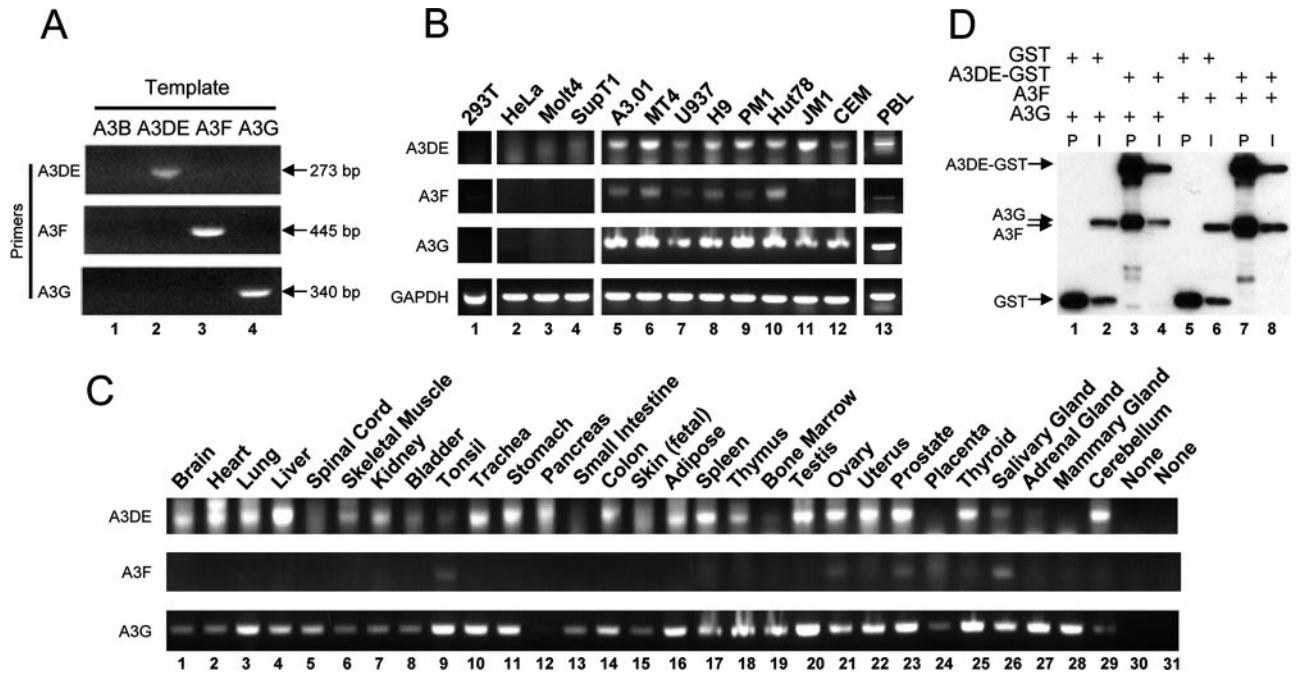

FIG. 5. Distribution of A3 transcripts in vivo and interactions of A3DE with A3F or A3G. (A) Specificity of the PCR primer pairs for A3DE, A3F, and A3G. One previously reported A3G primer pair (2) and two newly designed A3DE and A3F primer pairs were used to amplify A3DE, A3F, or A3G by use of their expression plasmids as templates. (B) Distribution of A3 transcripts in a variety of human cell lines and PBL. Total RNAs from these cells were subjected to RT-PCR with A3DE-, A3F-, and A3G-specific primers, and amplified PCR products were visualized by agarose gel electrophoresis followed by ethidium bromide staining. GAPDH was used as an internal control to compare each RNA input. (C) Distribution of A3 transcripts in a variety of human tissues. The same primer pairs described above were used to amplify A3DE, A3F, and A3G messengers from a commercially obtained prenormalized human multiple tissue cDNA panel by RT-PCR, and PCR products were analyzed similarly to the procedure described above. (D) A3DE interacts with A3F and A3G to form heteromultimers in cells. This experiment is similar to that described in the legend for Fig. 4B. The GST or A3DE-GST expression vector was cotransfected with the A3F or A3G expression vector into 293T cells. After 48 h, proteins were pulled down by GSH-Sepharose beads and analyzed by Western blotting with a V5 antibody.

To gain further insight into A3DE expression in vivo, we then determined A3 protein expression in human primary tissues (Fig. 5C). In 29 different human tissue-derived prenormalized cDNA samples, A3G was the most broadly expressed and was absent only in pancreas (Fig. 5C, lane 12). A3F was the least broadly expressed, appearing only in tonsil, ovary, prostate, and salivary gland (Fig. 5C, lanes 9, 21, 23, and 26). A3DE was expressed much more broadly than A3F and exhibited an expression profile very similar to that of A3G, although A3DE was not detected in spinal cord, small intestine, skin, placenta, or mammary gland (Fig. 5C, lanes 5, 13, 15, 24, and 28). To further confirm this observation, we determined A3 protein expression by a BLAST search of the serial analysis of gene expression (SAGE) database at the Cancer Genome Anatomy Project (CGAP) website. This program could not distinguish between expression of A3B and that of A3DE. However, since A3B has not been detected in any human tissues so far (2, 14), we could assume that our search results for A3B or A3DE expression represented only that of A3DE. As presented in Table 1, in normal human tissues, a total of 60, 18, and 76 copies were identified for A3DE, A3F, and A3G, respectively, which confirms the lower expression of A3F in vivo. Intriguingly, A3DE and A3G were found mostly in those white blood cells that are the same cell population as PBL, where A3F was less represented. This result was also consistent with our result shown in Fig. 5B, where A3F was less expressed

TABLE 1. Distribution of SAGE tags of different A3 proteins in normal human tissues[a]

Tissue	Copy no. of SAGE tags of protein:		
	A3B or A3DE	A3F	A3G
Bone marrow	7	1	2
Brain	0	1	4
Heart	0	1	1
Liver	0	0	0
Lung	0	0	1
Mammary gland	1	1	0
Ovary	1	0	0
Pancreas	1	0	0
Placenta	0	1	0
Prostate	0	1	0
Retina	0	0	3
Stem cell	15	8	4
Stomach	1	0	0
Thyroid	1	1	0
Vascular	0	2	2
White blood cells	33	1	59
Total	60	18	76

[a] GenBank accession numbers NM_004900, NM_152426, BC038808, and BC024268 for A3B, A3DE, A3F, and A3G, respectively, were used for a BLAST search of the SAGE tag expression database from the Cancer Genome Anatomy Project (CGAP) (http://cgap.nci.nih.gov/SAGE). Due to technical issues, this program did not distinguish between expression of A3B and that of A3DE.

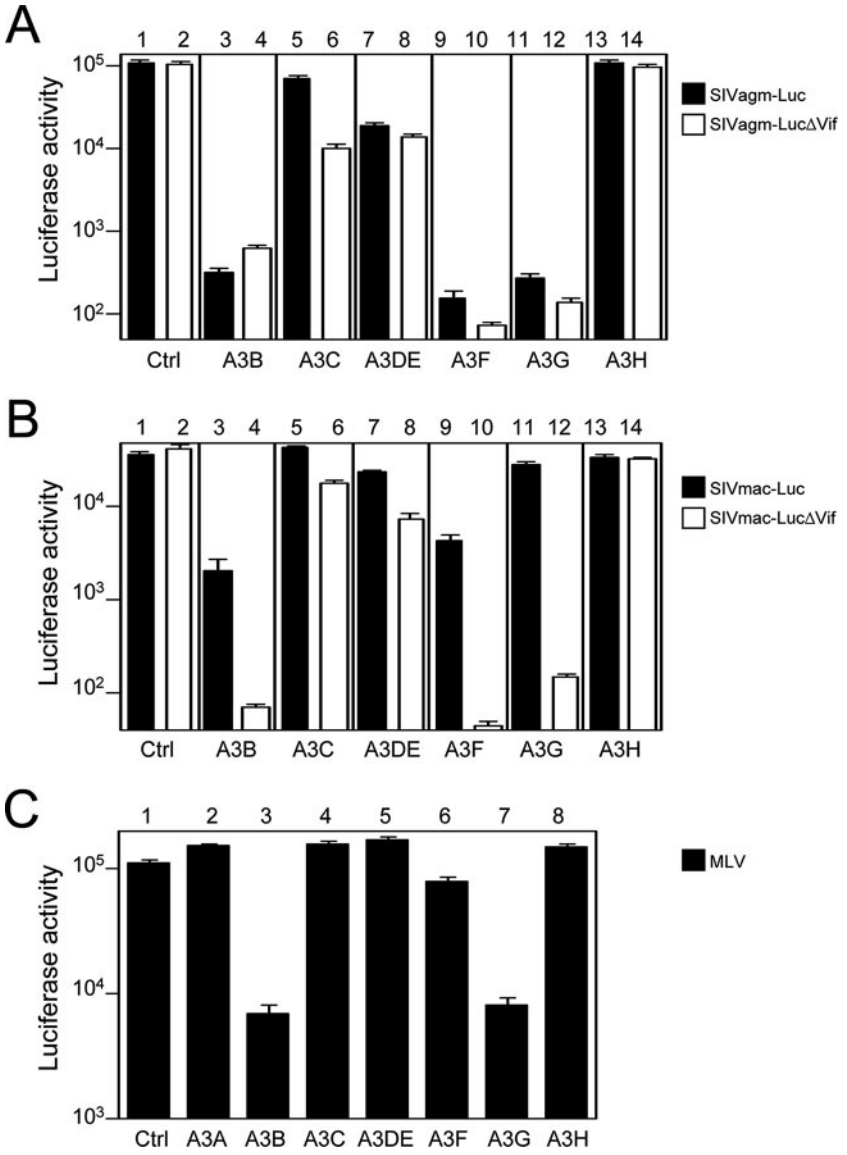

FIG. 6. A3DE blocks the replication of SIVagm and SIVmac but not that of MLV. Wild-type and Vif-defective (ΔVif) SIVagm (A) and SIVmac (B) or wild-type MLV (C) luciferase reporter viruses were produced from 293T cells in the presence or absence of the indicated human A3 proteins. After normalization by $p27^{Gag}$ enzyme-linked immunosorbent assay or RT activity, viruses were used to infect GHOST cells, and intracellular luciferase activities were quantified after 36 h of infection. Error bars represent the standard deviations from at least three independent experiments. Ctrl, control.

than A3DE and A3G in PBL (Fig. 5B, lane 13). Thus, A3DE and A3G were expressed much more broadly and abundantly than A3F in human tissues, especially in those primary targets for HIV-1 infection. Such a tissue-specific expression pattern may suggest different engagements of these genes in antiretroviral defense.

Although they were expressed differently, A3DE, A3F, and A3G were clearly coexpressed in certain cell lines and tissues (Fig. 5B and C). A3F and A3G have been reported previously to form heteromultimers (61), and such interaction may coordinate and magnify their antiretroviral effect. To know whether A3DE interacts with A3F and A3G, A3DE-GST (or its control GST) was coexpressed with A3F or A3G, and protein complexes were pulled down by GSH-Sepharose beads. As presented in Fig. 5D, both A3F and A3G were pulled down by A3DE-GST (Fig. 5D, lanes 2 and 6) but not by GST (Fig. 5D, lanes 1 and 5). We therefore conclude that A3DE and A3F or A3DE and A3G bind to each other to form heteromultimers when they are expressed in the same cells.

A3DE blocks SIV but not MLV replication. After showing that A3DE inhibited HIV-1 replication, we next asked whether A3DE could block other retroviruses, such as SIV and MLV. To simplify the detection of viral replication, similar luciferase reporter proviruses from SIV and MLV were used. In addition, several other human A3 genes were included in this study to serve as controls. Consistent with prior reports, we found that A3B inhibited the replication of both wild-type and Vif-defective SIVagm, SIVmac, and MLV; A3C inhibited the replica-

tion of Vif-defective SIVagm and SIVmac; A3F and A3G inhibited the replication of Vif-defective SIVagm and SIVmac and wild-type SIVagm; and A3G inhibited MLV replication (Fig. 6A, B, and C). We found that A3H did not block replication of any of these viruses and that A3DE did not block MLV replication. However, A3DE reduced the infectivity of both wild-type and Vif-defective SIVagm by approximately six- to eightfold and it also reduced the infectivity of Vif-defective SIVmac by sixfold (Fig. 6A, B, and C). Although the activity of A3DE was weaker than that of A3F or A3G, it exhibited antiretroviral activity that was comparable to (in the case of SIVagm) or higher than (in the case of SIVmac) that of A3C. Thus, A3DE blocked the replication of both SIVagm and SIVmac, whereas its activity was blocked by Vif from SIVmac but not SIVagm.

DISCUSSION

In this report, we have studied the antiretroviral activities of two human A3 proteins, A3DE and A3H. A3DE inhibits the replication of HIV-1, SIVagm, and SIVmac but not that of MLV. In contrast, A3H did not block any viral replication, as reported by another group very recently (44). A3DE was encapsidated to deaminate cytosines on viral minus-strand DNA in the target cells when Vif was absent. However, when Vif was present, A3DE was polyubiquitylated by Cul5-based E3 ligases and degraded in the 26S proteasome. A3DE was expressed much more extensively than A3F in nonpermissive cells and tissues and formed heteromultimers with A3G or A3F. These results suggest that A3DE may play a prominent role in synchronizing with A3G to block HIV-1 or SIV infection.

Human A3B, A3DE, A3F, and A3G, which all block HIV-1 replication, share an important molecular structure: two Zn^{2+}-binding motifs separated in their N- and C-terminal regions. Although mutational analysis of A3G showed that both motifs were required for its anti-HIV-1 activity (35, 71), these motifs have different functions. The first Zn^{2+}-binding motif was shown to determine RNA binding and virion incorporation of A3G, which suggests that it is involved in the substrate recognition of the enzyme (42). On the other hand, the second Zn^{2+}-binding motif was shown to not only determine the enzymatic activity of A3G but also govern the target sequence specificities of both A3F and A3G (19, 28, 43). Thus, the second Zn^{2+}-binding motif contributes more directly to A3 antiretroviral activity. In fact, our results provide another piece of evidence to support the important role of the second Zn^{2+}-binding motif of A3 proteins in their antiretroviral activities. As shown in Fig. 2, 3, and 6, both A3DE and A3F inhibited the replication of HIV-1 and SIV but not that of MLV and they both introduced GA-to-AA mutations on the HIV-1 plus-strand DNA. Indeed, the C-terminal halves of A3DE and A3F are almost identical (Fig. 1) and such genomic similarity might be the determinant for their quite similar antiretroviral profiles.

Another important observation in this report is that A3DE was expressed more extensively than A3F in nonpermissive cells and tissues. Previously, it was found that although A3B, A3F, and A3G blocked HIV replication, they were expressed differently. A3F and A3G were coexpressed in nonpermissive T cells and many tissues, including PBL (2, 30, 61), whereas A3B was not detected at all (14). Nonetheless, using the same RT-PCR strategy, we found that those previously detected A3F mRNAs might also include those from A3DE. In distinguishing these two mRNA species, we found that A3DE was expressed more broadly and abundantly than A3F in nonpermissive cells and tissues. This was seen especially with PBL. Furthermore, it was reported that the HIV-1 genome experienced a high number of G-to-A hypermutations in vivo, including GG-to-AG, GA-to-AA, GC-AC, and GT-to-AT mutations, whereas the GG-to-AG and GA-to-AA mutations were attributed to A3G and A3F, respectively (51). As shown in Fig. 3B, A3DE introduces both GA-to-AA and GC-to-AC mutations to the HIV-1 genome. Thus, the existence of these GA-to-AA and GC-to-AC mutations from HIV-1 clinical isolates provides important evidence for A3DE activity in vivo. It therefore seems to be possible that A3DE might play a more important role than A3F in complementing the intracellular immunity to HIV-1 by A3G, which has been attributed solely to A3F before.

It is still puzzling why primates encode so many duplicative A3 genes, some of which share the same targets. The existence of such paralogous genes seems functionally redundant, but comparative studies of the antiviral activities of these A3 orthologous genes in humans and monkeys have provided some key implications for understanding this genomic complexity. First, it was shown before that, although A3F exhibited much lower activity to HIV-1 than A3G in humans, this phenotype was reversed in monkeys, where A3F exhibited even higher activity than A3G, which suggests that simian A3F preferentially targets HIV-1 instead of SIV (68). Second, although human A3H did not block any viral replication, macaque A3H efficiently blocked the replication of both SIV and HIV-1 (44). On the other hand, although human A3A and A3C did not block HIV-1, they effectively inhibited LINE-1/IAP retrotransposons and SIV, respectively (4, 64). Thus, each A3 gene clearly has distinctive primary targets in different species so that they can interweave different layers of a defense network to defeat various retrovirus infections.

Unfortunately, lentiviruses, except equine infectious anemia virus, ultimately succeed in infection by expression of Vif, a protein that is able to knock out most of these A3 proteins. Vif has two interfaces to interact with ElonginC and Cul5, which form the core units for the Cul5-based E3 ubiquitin ligase complex. In addition, it interacts with A3DE, A3F, and A3G. The mechanism by which Vif binds to these different A3 proteins remains enigmatic. It seems unlikely that these proteins share similar surfaces that can be recognized by Vif, because a similar negatively charged residue (D128) that affected sensitivity to Vif in A3G did not play such a role in A3DE and A3F. More likely, these interactions might not be a direct event and may be mediated by cellular RNA, because both Vif and A3 proteins have the capability to bind to RNA (13, 21, 22, 24, 65, 70). Alternatively, Vif may have additional different interfaces to bind to these different A3 proteins, as suggested by a recent study (58). Nonetheless, understanding these mechanisms will be very helpful for the design of inhibitors that disrupt Vif and A3 interactions, which is of great medicinal importance in HIV/AIDS therapeutics (10, 54).

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REFERENCES

1. **Alce, T. M., and W. Popik.** 2004. APOBEC3G is incorporated into virus-like particles by a direct interaction with HIV-1 Gag nucleocapsid protein. J. Biol. Chem. **279:**34083–34086.
2. **Bishop, K. N., R. K. Holmes, A. M. Sheehy, N. O. Davidson, S. J. Cho, and M. H. Malim.** 2004. Cytidine deamination of retroviral DNA by diverse APOBEC proteins. Curr. Biol. **14:**1392–1396.
3. **Bogerd, H. P., B. P. Doehle, H. L. Wiegand, and B. R. Cullen.** 2004. A single amino acid difference in the host APOBEC3G protein controls the primate species specificity of HIV type 1 virion infectivity factor. Proc. Natl. Acad. Sci. USA **101:**3770–3774.
4. **Bogerd, H. P., H. L. Wiegand, B. P. Doehle, K. K. Lueders, and B. R. Cullen.** 2006. APOBEC3A and APOBEC3B are potent inhibitors of LTR-retrotransposon function in human cells. Nucleic Acids Res. **34:**89–95.
5. **Bogerd, H. P., H. L. Wiegand, A. E. Hulme, J. L. Garcia-Perez, K. S. O'Shea, J. V. Moran, and B. R. Cullen.** 2006. Cellular inhibitors of long interspersed element 1 and Alu retrotransposition. Proc. Natl. Acad. Sci. USA **103:**8780–8785.
6. **Bour, S., and K. Strebel.** 2000. HIV accessory proteins: multifunctional components of a complex system. Adv. Pharmacol. **48:**75–120.
7. **Butler, S. L., M. S. Hansen, and F. D. Bushman.** 2001. A quantitative assay for HIV DNA integration in vivo. Nat. Med. **7:**631–634.
8. **Cen, S., F. Guo, M. Niu, J. Saadatmand, J. Deflassieux, and L. Kleiman.** 2004. The interaction between HIV-1 Gag and APOBEC3G. J. Biol. Chem. **279:**33177–33184.
9. **Chen, H., C. E. Lilley, Q. Yu, D. V. Lee, J. Chou, I. Narvaiza, N. R. Landau, and M. D. Weitzman.** 2006. APOBEC3A is a potent inhibitor of adeno-associated virus and retrotransposons. Curr. Biol. **16:**480–485.
10. **Chiu, Y. L., and W. C. Greene.** 2006. APOBEC3 cytidine deaminases: distinct antiviral actions along the retroviral life cycle. J. Biol. Chem. **281:**8309–8312.
11. **Conticello, S. G., C. J. Thomas, S. K. Petersen-Mahrt, and M. S. Neuberger.** 2005. Evolution of the AID/APOBEC family of polynucleotide (deoxy)-cytidine deaminases. Mol. Biol. Evol. **22:**367–377.
12. **Cullen, B. R.** 2006. Role and mechanism of action of the APOBEC3 family of antiretroviral resistance factors. J. Virol. **80:**1067–1076.
13. **Dettenhofer, M., S. Cen, B. A. Carlson, L. Kleiman, and X.-F. Yu.** 2000. Association of human immunodeficiency virus type 1 Vif with RNA and its role in reverse transcription. J. Virol. **74:**8938–8945.
14. **Doehle, B. P., A. Schafer, and B. R. Cullen.** 2005. Human APOBEC3B is a potent inhibitor of HIV-1 infectivity and is resistant to HIV-1 Vif. Virology **339:**281–288.
15. **Doehle, B. P., A. Schafer, H. L. Wiegand, H. P. Bogerd, and B. R. Cullen.** 2005. Differential sensitivity of murine leukemia virus to APOBEC3-mediated inhibition is governed by virion exclusion. J. Virol. **79:**8201–8207.
16. **Douaisi, M., S. Dussart, M. Courcoul, G. Bessou, R. Vigne, and E. Decroly.** 2004. HIV-1 and MLV Gag proteins are sufficient to recruit APOBEC3G into virus-like particles. Biochem. Biophys. Res. Commun. **321:**566–573.
17. **Gabuzda, D. H., K. Lawrence, E. Langhoff, E. Terwilliger, T. Dorfman, W. A. Haseltine, and J. Sodroski.** 1992. Role of *vif* in replication of human immunodeficiency virus type 1 in $CD4^+$ T lymphocytes. J. Virol. **66:**6489–6495.
18. **Goff, S., P. Traktman, and D. Baltimore.** 1981. Isolation and properties of Moloney murine leukemia virus mutants: use of a rapid assay for release of virion reverse transcriptase. J. Virol. **38:**239–248.
19. **Hache, G., M. T. Liddament, and R. S. Harris.** 2005. The retroviral hypermutation specificity of APOBEC3F and APOBEC3G is governed by the C-terminal DNA cytosine deaminase domain. J. Biol. Chem. **280:**10920–10924.
20. **Harris, R. S., K. N. Bishop, A. M. Sheehy, H. M. Craig, S. K. Petersen-Mahrt, I. N. Watt, M. S. Neuberger, and M. H. Malim.** 2003. DNA deamination mediates innate immunity to retroviral infection. Cell **113:**803–809.
21. **Henriet, S., D. Richer, S. Bernacchi, E. Decroly, R. Vigne, B. Ehresmann, C. Ehresmann, J. C. Paillart, and R. Marquet.** 2005. Cooperative and specific binding of Vif to the 5′ region of HIV-1 genomic RNA. J. Mol. Biol. **354:**55–72.
22. **Jarmuz, A., A. Chester, J. Bayliss, J. Gisbourne, I. Dunham, J. Scott, and N. Navaratnam.** 2002. An anthropoid-specific locus of orphan C to U RNA-editing enzymes on chromosome 22. Genomics **79:**285–296.
23. **Karczewski, M. K., and K. Strebel.** 1996. Cytoskeleton association and virion incorporation of the human immunodeficiency virus type 1 Vif protein. J. Virol. **70:**494–507.
24. **Khan, M. A., C. Aberham, S. Kao, H. Akari, R. Gorelick, S. Bour, and K. Strebel.** 2001. Human immunodeficiency virus type 1 Vif protein is packaged into the nucleoprotein complex through an interaction with viral genomic RNA. J. Virol. **75:**7252–7265.
25. **Khan, M. A., S. Kao, E. Miyagi, H. Takeuchi, R. Goila-Gaur, S. Opi, C. L. Gipson, T. G. Parslow, H. Ly, and K. Strebel.** 2005. Viral RNA is required for the association of APOBEC3G with human immunodeficiency virus type 1 nucleoprotein complexes. J. Virol. **79:**5870–5874.
26. **Kobayashi, M., A. Takaori-Kondo, K. Shindo, A. Abudu, K. Fukunaga, and T. Uchiyama.** 2004. APOBEC3G targets specific virus species. J. Virol. **78:**8238–8244.
27. **Kremer, M., A. Bittner, and B. S. Schnierle.** 2005. Human APOBEC3G incorporation into murine leukemia virus particles. Virology **337:**175–182.
28. **Langlois, M. A., R. C. Beale, S. G. Conticello, and M. S. Neuberger.** 2005. Mutational comparison of the single-domained APOBEC3C and double-domained APOBEC3F/G anti-retroviral cytidine deaminases provides insight into their DNA target site specificities. Nucleic Acids Res. **33:**1913–1923.
29. **Lecossier, D., F. Bouchonnet, F. Clavel, and A. J. Hance.** 2003. Hypermutation of HIV-1 DNA in the absence of the Vif protein. Science **300:**1112.
30. **Liddament, M. T., W. L. Brown, A. J. Schumacher, and R. S. Harris.** 2004. APOBEC3F properties and hypermutation preferences indicate activity against HIV-1 in vivo. Curr. Biol. **14:**1385–1391.
31. **Liu, B., P. T. N. Sarkis, K. Luo, Y. Yu, and X.-F. Yu.** 2005. Regulation of Apobec3F and human immunodeficiency virus type 1 Vif by Vif-Cul5-ElonB/C E3 ubiquitin ligase. J. Virol. **79:**9579–9587.
32. **Luo, K., B. Liu, Z. Xiao, Y. Yu, X. Yu, R. Gorelick, and X.-F. Yu.** 2004. Amino-terminal region of the human immunodeficiency virus type 1 nucleocapsid is required for human APOBEC3G packaging. J. Virol. **78:**11841–11852.
33. **Luo, K., Z. Xiao, E. Ehrlich, Y. Yu, B. Liu, S. Zheng, and X. F. Yu.** 2005. Primate lentiviral virion infectivity factors are substrate receptors that assemble with cullin 5-E3 ligase through a HCCH motif to suppress APOBEC3G. Proc. Natl. Acad. Sci. USA **102:**11444–11449.
34. **MacGinnitie, A. J., S. Anant, and N. O. Davidson.** 1995. Mutagenesis of apobec-1, the catalytic subunit of the mammalian apolipoprotein B mRNA editing enzyme, reveals distinct domains that mediate cytosine nucleoside deaminase, RNA binding, and RNA editing activity. J. Biol. Chem. **270:**14768–14775.
35. **Mangeat, B., P. Turelli, G. Caron, M. Friedli, L. Perrin, and D. Trono.** 2003. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. Nature **424:**99–103.
36. **Mangeat, B., P. Turelli, S. Liao, and D. Trono.** 2004. A single amino acid determinant governs the species-specific sensitivity of APOBEC3G to Vif action. J. Biol. Chem. **279:**14481–14483.
37. **Mariani, R., D. Chen, B. Schrofelbauer, F. Navarro, R. Konig, B. Bollman, C. Munk, H. Nymark-McMahon, and N. R. Landau.** 2003. Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. Cell **114:**21–31.
38. **Marin, M., K. M. Rose, S. L. Kozak, and D. Kabat.** 2003. HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. Nat. Med. **9:**1398–1403.
39. **Mehle, A., J. Goncalves, M. Santa-Marta, M. McPike, and D. Gabuzda.** 2004. Phosphorylation of a novel SOCS-box regulates assembly of the HIV-1 Vif-Cul5 complex that promotes APOBEC3G degradation. Genes Dev. **18:**2861–2866.
40. **Mikl, M. C., I. N. Watt, M. Lu, W. Reik, S. L. Davies, M. S. Neuberger, and C. Rada.** 2005. Mice deficient in APOBEC2 and APOBEC3. Mol. Cell. Biol. **25:**7270–7277.
41. **Muramatsu, M., V. S. Sankaranand, S. Anant, M. Sugai, K. Kinoshita, N. O. Davidson, and T. Honjo.** 1999. Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. J. Biol. Chem. **274:**18470–18476.
42. **Navarro, F., B. Bollman, H. Chen, R. Konig, Q. Yu, K. Chiles, and N. R.**

Landau. 2005. Complementary function of the two catalytic domains of APOBEC3G. Virology **333:**374–386.
43. **Newman, E. N., R. K. Holmes, H. M. Craig, K. C. Klein, J. R. Lingappa, M. H. Malim, and A. M. Sheehy.** 2005. Antiviral function of APOBEC3G can be dissociated from cytidine deaminase activity. Curr. Biol. **15:**166–170.
44. **OhAinle, M., J. A. Kerns, H. S. Malik, and M. Emerman.** 2006. Adaptive evolution and antiviral activity of the conserved mammalian cytidine deaminase APOBEC3H. J. Virol. **80:**3853–3862.
45. **Rogozin, I. B., M. K. Basu, I. K. Jordan, Y. I. Pavlov, and E. V. Koonin.** 2005. APOBEC4, a new member of the AID/APOBEC family of polynucleotide (deoxy)cytidine deaminases predicted by computational analysis. Cell Cycle **4:**1281–1285.
46. **Rose, K. M., M. Marin, S. L. Kozak, and D. Kabat.** 2005. Regulated production and anti-HIV type 1 activities of cytidine deaminases APOBEC3B, 3F, and 3G. AIDS Res. Hum. Retrovir. **21:**611–619.
47. **Schafer, A., H. P. Bogerd, and B. R. Cullen.** 2004. Specific packaging of APOBEC3G into HIV-1 virions is mediated by the nucleocapsid domain of the gag polyprotein precursor. Virology **328:**163–168.
48. **Schrofelbauer, B., D. Chen, and N. R. Landau.** 2004. A single amino acid of APOBEC3G controls its species-specific interaction with virion infectivity factor (Vif). Proc. Natl. Acad. Sci. USA **101:**3927–3932.
49. **Sheehy, A. M., N. C. Gaddis, J. D. Choi, and M. H. Malim.** 2002. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature **418:**646–650.
50. **Sheehy, A. M., N. C. Gaddis, and M. H. Malim.** 2003. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. Nat. Med. **9:**1404–1407.
51. **Simon, V., V. Zennou, D. Murray, Y. Huang, D. D. Ho, and P. D. Bieniasz.** 2005. Natural variation in Vif: differential impact on APOBEC3G/3F and a potential role in HIV-1 diversification. PLoS Pathog. **1:**e6.
52. **Stenglein, M. D., and R. S. Harris.** 2006. APOBEC3B and APOBEC3F inhibit L1 retrotransposition by a DNA deamination-independent mechanism. J. Biol. Chem. **281:**16837–16841.
53. **Stopak, K., C. de Noronha, W. Yonemoto, and W. C. Greene.** 2003. HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability. Mol. Cell **12:**591–601.
54. **Stopak, K., and W. C. Greene.** 2005. Protecting APOBEC3G: a potential new target for HIV drug discovery. Curr. Opin. Investig. Drugs **6:**141–147.
55. **Strebel, K., D. Daugherty, K. Clouse, D. Cohen, T. Folks, and M. A. Martin.** 1987. The HIV 'A' (sor) gene product is essential for virus infectivity. Nature **328:**728–730.
56. **Svarovskaia, E. S., H. Xu, J. L. Mbisa, R. Barr, R. J. Gorelick, A. Ono, E. O. Freed, W. S. Hu, and V. K. Pathak.** 2004. Human apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G) is incorporated into HIV-1 virions through interactions with viral and nonviral RNAs. J. Biol. Chem. **279:**35822–35828.
57. **Teng, B., C. F. Burant, and N. O. Davidson.** 1993. Molecular cloning of an apolipoprotein B messenger RNA editing protein. Science **260:**1816–1819.
58. **Tian, C., X. Yu, W. Zhang, T. Wang, R. Xu, and X.-F. Yu.** 2006. Differential requirement for conserved tryptophans in human immunodeficiency virus type 1 Vif for the selective suppression of APOBEC3G and APOBEC3F. J. Virol. **80:**3112–3115.
59. **von Schwedler, U., J. Song, C. Aiken, and D. Trono.** 1993. Vif is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells. J. Virol. **67:**4945–4955.
60. **Wedekind, J. E., G. S. Dance, M. P. Sowden, and H. C. Smith.** 2003. Messenger RNA editing in mammals: new members of the APOBEC family seeking roles in the family business. Trends Genet. **19:**207–216.
61. **Wiegand, H. L., B. P. Doehle, H. P. Bogerd, and B. R. Cullen.** 2004. A second human antiretroviral factor, APOBEC3F, is suppressed by the HIV-1 and HIV-2 Vif proteins. EMBO J. **23:**2451–2458.
62. **Xu, H., E. S. Svarovskaia, R. Barr, Y. Zhang, M. A. Khan, K. Strebel, and V. K. Pathak.** 2004. A single amino acid substitution in human APOBEC3G antiretroviral enzyme confers resistance to HIV-1 virion infectivity factor-induced depletion. Proc. Natl. Acad. Sci. USA **101:**5652–5657.
63. **Yamanaka, S., K. S. Poksay, M. E. Balestra, G. Q. Zeng, and T. L. Innerarity.** 1994. Cloning and mutagenesis of the rabbit ApoB mRNA editing protein. A zinc motif is essential for catalytic activity, and noncatalytic auxiliary factor(s) of the editing complex are widely distributed. J. Biol. Chem. **269:**21725–21734.
64. **Yu, Q., D. Chen, R. Konig, R. Mariani, D. Unutmaz, and N. R. Landau.** 2004. APOBEC3B and APOBEC3C are potent inhibitors of simian immunodeficiency virus replication. J. Biol. Chem. **279:**53379–53386.
65. **Yu, Q., R. Konig, S. Pillai, K. Chiles, M. Kearney, S. Palmer, D. Richman, J. M. Coffin, and N. R. Landau.** 2004. Single-strand specificity of APOB EC3G accounts for minus-strand deamination of the HIV genome. Nat. Struct. Mol. Biol. **11:**435–442.
66. **Yu, X., Y. Yu, B. Liu, K. Luo, W. Kong, P. Mao, and X. F. Yu.** 2003. Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. Science **302:**1056–1060.
67. **Yu, Y., Z. Xiao, E. S. Ehrlich, X. Yu, and X. F. Yu.** 2004. Selective assembly of HIV-1 Vif-Cul5-ElonginB-ElonginC E3 ubiquitin ligase complex through a novel SOCS box and upstream cysteines. Genes Dev. **18:**2867–2872.
68. **Zennou, V., and P. D. Bieniasz.** 2006. Comparative analysis of the antiretroviral activity of APOBEC3G and APOBEC3F from primates. Virology **349:**31–40.
69. **Zennou, V., D. Perez-Caballero, H. Gottlinger, and P. D. Bieniasz.** 2004. APOBEC3G incorporation into human immunodeficiency virus type 1 particles. J. Virol. **78:**12058–12061.
70. **Zhang, H., R. J. Pomerantz, G. Dornadula, and Y. Sun.** 2000. Human immunodeficiency virus type 1 Vif protein is an integral component of an mRNP complex of viral RNA and could be involved in the viral RNA folding and packaging process. J. Virol. **74:**8252–8261.
71. **Zhang, H., B. Yang, R. J. Pomerantz, C. Zhang, S. C. Arunachalam, and L. Gao.** 2003. The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. Nature **424:**94–98.
72. **Zheng, Y. H., D. Irwin, T. Kurosu, K. Tokunaga, T. Sata, and B. M. Peterlin.** 2004. Human APOBEC3F is another host factor that blocks human immunodeficiency virus type 1 replication. J. Virol. **78:**6073–6076.